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PERSPECTIVE

Genomic Plasticity and the Diversity of Polyploid Plants

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Polyploidy, a change whereby the entire chromosome set is multiplied, arises through mitotic or meiotic misdivisions and frequently involves unreduced gametes and interspecific hybridization. The success of newly formed angiosperm polyploids is partly attributable to their highly plastic genome structure, as manifested by tolerance to changing chromosome numbers (aneuploidy and polyploidy), genome size, (retro)transposable element mobility, insertions, deletions, and epigenome restructuring. The ability to withstand large-scale changes, frequently within one or a few generations, is associated with a restructuring of the transcriptome, metabolome, and proteome and can result in an altered phenotype and ecology. Thus, polyploid-induced changes can generate individuals that are able to exploit new niches or to outcompete progenitor species. This process has been a major driving force behind the divergence of the angiosperms and their biodiversity.

Polyploidy occurs in many animals (e.g., in fishes, insects, and amphibians) and plants (e.g., ferns and mosses) but is particularly widespread in the flowering plants (angiosperms), including many major crops (Fig. 1). Molecular analyses suggest that the genomes of most (>90%) extant angiosperms retain evidence of one or more ancient genomewide duplications (*1*) and that numerous species have undergone more recent polyploidy (*2*). For example, the polyploid *Arabidopsis suecica* is considered to have formed from "diploid" parents circa (ca.) 12,000 to 300,000 years ago, whereas each of the parental genomes retain evidence of more ancient duplications estimated to have arisen ca. 101 to 168 million years ago (Ma), 66 to 109 Ma, and 24 to 40 Ma (*3*). Polyploidy often occurs in association with interspecific hybridization, a condition called allopolyploidy, that can result in the generation of new species. It is not clear why polyploidy is so abundant in angiosperms, particularly when its occurrence in gymnosperms, considered to be sister to angiosperms, is so low (<5% are polyploid). Nevertheless we wish to know the origin, evolution, and consequences of polyploidy to reveal how it has contributed to the diversity of angiosperms seen today (estimated to be 250,000 to 400,000 species) (*4*–*10*).

Polyploids commonly arise from unreduced gametes by nondisjunction of chromosomes in the germ line. Unreduced gametes occur widely in both animals and plants, but there are differences in the survival of the derived triploid offspring. For example, in humans diploid gametes occur 0.2 to 0.3% of the time, but they rarely lead to triploid young and these do not survive to adulthood. In angiosperms, unreduced gametes occur with a mean frequency of about 0.56%, with interspecific hybridization, herbivory, and disease stress, among others, elevating their occurrence (*11*). However, in contrast to humans, derived triploid angiosperms frequently survive and can be important in establishing higher ploidy levels (*12*). Furthermore, ongoing chromosome

Fig. 1. A sample of agricultural crops that are polyploid, showing oil from oilseed rape (*Brassica napus*, $2n = 4x = 38$), bread from bread wheat (*Triticum aestivum*, $2n = 6x = 42$), rope from sisal (*Agave sisalana*, $2n = 5x = 180$), coffee beans (*Coffea arabica*, $2n = 4x = 44$), banana (*Musa* triploid hybrids, $2n = 3x = 33$), cotton (*Gossypium hirsutum*, $2n = 4x = 52$), potatoes (*Solanum tuberosum*, $2n = 4x = 48$), and maize (*Zea mays*, $2n = 4x = 20$).

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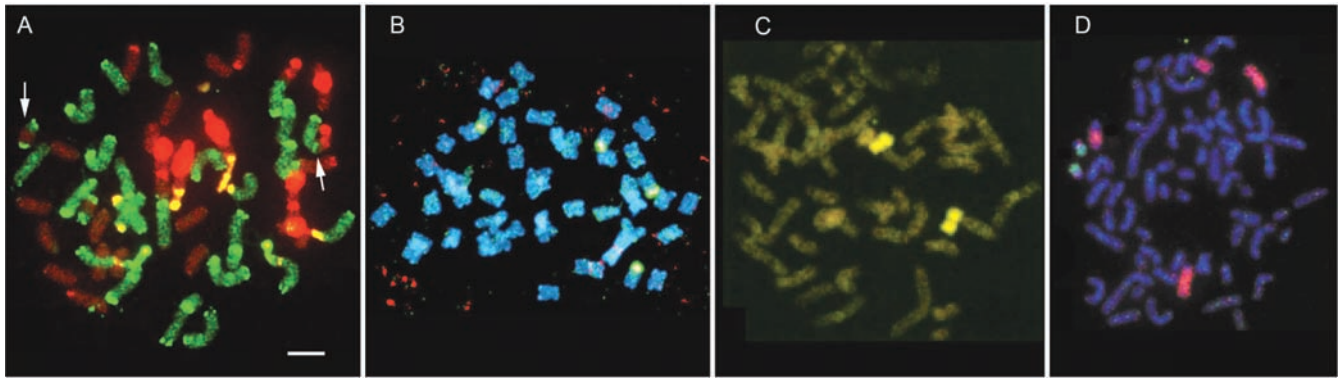

Fig. 2. Differences in genomic plasticity between angiosperms and mammals revealed by chromosome labeling patterns using fluorescent in situ hybridization. (**A**) Genome painting distinguishes parental genomes in polyploid synthetic tobacco ($2n = 4x = 48$, 1C = 5.4 pg). Chromosomes were labeled with both red and green fluorescent markers that identify the parental origin of the chromatin by color. Chromosomes with red and green segments carry translocations (arrows). [Image reproduced with permission from (*24*). Copyright 2006, Botanical Society of America] (**B**) The differential labeling of parental chromatin is lost in a related allopolyploid, *Nicotiana nesophila* ($2n = 4x = 48$, 1C = 5.0 pg), because of near-complete genome turnover occurring over ca. 5 million years. [Image reproduced with permission from (*25*). Copyright 2005, *New Phytologist*] In contrast, the conserved genome structure of mammals is illustrated by the ability of human chromosome paints to label chromosomes of (**C**) orangutan ($2n = 2x = 48$, 1C = 3.6 pg), which diverged from humans more than 15 Ma, and (**D**) horse ($2n = 2x = 64$, 1C = ca. 3.2 pg), which diverged over 55 Ma. [Image in (C) reproduced with permission from (*26*). Copyright 1992, National Academy of Sciences. Image in (D) reproduced with permission from (*27*). Copyright 2004, Springer-Verlag] Scale bars indicate 10 μm [(A) and (B)], ca. 3.5 μm (C), and 4.5 μm (D).

nondisjunction can give rise to complex aneuploid and polyploid series (e.g., *Cardamine pratensis* L., with reported chromosome numbers of $2n =$ 16, 24, 28, 30, 33 to 38, 40 to 46, 48, 52 to 64, and 67 to 96). Newly formed allopolyploids may be particularly common in angiosperms, given the abundance of unreduced gametes and the general lack of targeting in pollen delivery (e.g., via insects or wind) to the female receptive organ in the flower (the stigma). However, few successfully establish. Indeed, the allopolyploid plant York groundsel (*Senecio eboracensis*, Asteraceae) probably arose naturally in the 1970s through allopolyploidy involving a native and introduced species. It now grows as a weed of industrial wastelands, but redevelopment of the area is destroying its habitat, and unless it can disperse to similar sites elsewhere it will go extinct. Thus, in less than 50 years from its birth, this species is now nearing extinction, illustrating the precarious nature of early polyploid establishment (*5*). Nevertheless polyploid species are highly numerous, and even in the past 150 years several new species are known to have evolved and established (*6*).

Genomic Plasticity in Polyploids

Angiosperms are remarkable in their ability to tolerate the considerable genomic impact of polyploidy arising from the accommodation of divergent genomes in the same nucleus, intraspecific chromosome number variations, unbalanced parental chromatin contributions, and chromosomal rearrangements, for example, inversions and translocations (*13*) (examples of intergenomic translocations are arrowed in Fig. 2A, which shows a chromosome spread at metaphase of a polyploid species). This remarkable plasticity of the genome is also evident from the diverse and often rapid genetic and epigenetic changes associated with polyploidy [e.g., (retro)transposon mobility, sequence rearrangements and losses, gene silencing, DNA methylation changes, and chromatin remodeling (*2*, *6*, *8*)]. Many chromosomal changes are induced by multivalent formation and aberrant segregation of chromosomes at meiosis in early generation polyploids (*10*). These abnormalities can reduce fertility, but sexual selection will favor the most fertile and viable individuals and remove those that are maladapted. Little is known about how regular chromosome pairing is restored, although pairing genes may enhance bivalent formation in some polyploid wheats and mustards. Meiosis may therefore dually impact the evolution of many newly formed polyploids by (i) enabling sexual propagation and (ii) generating, through meiotic errors, large-scale chromosomal variation upon which genetic drift and/or selection can act. Overall, we surmise that the high frequencies of unreduced gametes provide a constant evolutionary pressure toward polyploidy in angiosperms, whereas genomic plasticity relaxes the genetic and developmental constraints against polyploid formation.

Studies of synthetic allopolyploids, aiming to mimic natural species, have been valuable in providing insights into the nature of angiosperm genome plasticity. They reveal that the genomic response to polyploidy can be fast, targeted, but highly variable between species (*8*). Nevertheless, changes in the epigenetic profile appears to be a universal response that can lead to partitioning of the expression of duplicated ancestral genes to specialized tissue-specific activity or function (subfunctionalization) (*2*). Duplicate genes can, however, also be lost, and over longer time frames this contributes to genome diploidization, an ongoing process that returns the genome to a diploidlike form (*14*).

In angiosperms, genomewide turnover of nongenic and/or repetitive sequences leads to further diploidization through the erosion of differences between parental genomes in the polyploid. Over time frames of 5 to 10 million years there can be near-complete retroelement turnover (*15*) and tandem repeat replacement, resulting in the loss of many genome-specific sequences (*16*) (Fig. 2, A and B). This genome plasticity contrasts markedly with mammalian genomes, in which single chromosome losses or gains are usually detrimental, polyploidy is rare or absent, and large-scale genome organization remains conserved over tens of millions of years (Fig. 2, C and D).

With high-throughput DNA sequencing technologies, it is now feasible to determine the parental origin of sequences in both the genomic DNA and the transcriptome and to compare profiles between natural and synthetic polyploids and related diploids. Such approaches will inform the extent to which differential sequence losses are targeted at specific genomes, chromosomes, or chromosomal regions; the influence of maternal and paternal origins; the role of environmental factors; and the mechanisms and processes involved in the evolution of duplicated alleles.

Genome Size Plasticity

Angiosperm genome sizes vary nearly 2000-fold compared with taxa that generally lack polyploidy, such as mammals (genome size varies fivefold) and birds (genome size varies twofold), emphasizing the plasticity in angiosperm genome structure. Polyploidy results in an instantaneous multiplication in DNA content, after which di-

vergence of lineages may be accompanied by genome size increases or decreases, the direction of which reflects a balance between mechanisms that expand genomes (e.g., retroelement insertion) and those which shrink them (e.g., deletions). With the accumulation of extra genome(s), there is an increased demand for nutrients, particularly nitrates and phosphates, that are needed to make nucleic acids and proteins (*17*). Yet phosphorus is often limited in supply, as in Australia, where ancient weathered soils have so little phosphorus that they restrict the distribution of some plant communities (*18*). Such nutrient demands are not a problem for animals that assimilate DNA directly from their food. However, for plants in areas where nutrient levels are limiting, we predict that there will be selection against polyploids or for those that can eliminate excess DNA. This hypothesis is supported by the fact that after polyploidy, genome downsizing in angiosperms is the most common response (*17*). More research is needed to determine the role of nutrient limitation in the establishment and evolution of polyploids in comparison with related diploids and to examine the incidence of polyploidy in those environments, such as bogs and heaths, where nutrient levels are low. One might also predict that similar trends toward genome downsizing would be less apparent in animals for which such nutrients are not limiting, but to date no comparable analyses have been done.

Transcriptome, Metabolome, and Proteome

Genomic plasticity has downstream effects on the transcriptome, proteome, and metabolome that can generate phenotypic variation in polyploids exceeding that found in the parents (*9*). At the transcriptome level, studies on natural and synthetic polyploids have demonstrated genomewide nonadditive, nonrandom changes in gene regulation (e.g., silencing and up- and down-regulation), many of which were tissue and/or species-specific. The reprogramming of the allopolyploid transcriptome has been shown to be triggered predominantly by interspecific hybridization rather than by chromosome multiplication (*9*).

Changes at the transcriptome need not necessarily be reflected in the proteome, given the potential for posttranscriptional and posttranslational modifications. Unfortunately, there are few studies into the effects of polyploidy on the proteome. Nevertheless, research on synthetic *Brassica* allopolyploids showed that 25 to 38% (depending on tissue) of proteins displayed quantitative variation from an expected additive pattern of proteins found in the two parents, with changes being rapid and, in some instances, nonstochastic, reproducible, and organ-specific (less than 1% of changes were qualitative). As with the transcriptome, it was interspecific hybridization that stimulated the most perturbations in expression (*19*). Interestingly, in silico analysis of nonadditive proteins failed to reveal any substantial biological consequences (*20*).

Not surprisingly, allopolyploidy has also been shown to trigger changes to the metabolome. The profile of secondary metabolites manufactured in defense of herbivory were shown to differ substantially in response time, duration, and strength between two *Nicotiana* allopolyploids that share the same parental origins (*21*). Similarly, in synthetic *Nicotiana* allopolyploids differences were observed between individuals and their parents (*22*). In nature, such physiological differences may affect allopolyploid evolution and survival.

The picture emerging from studies of synthetic polyploids is that interspecific hybridization is primarily responsible for triggering extensive and rapid changes and generating variation that may facilitate adaptation and speciation. Given this, one might expect homoploid species, (i.e., those derived from interspecific hybrids without an accompanying genome duplication) to be more common, yet only about 20 have been reported. Homoploids exploit niches unavailable to the parents and may diverge through spatial isolation or rapid genome divergence. In some cases, their success has been attributed to the acquisition of transgressive characters, that is, those characters that fall outside the range found in the parents (*23*). However, interspecific hybridization typically causes gene flow between populations, breaking down species isolation barriers, and does not usually lead to speciation. In contrast, chromosome multiplication can create instant barriers with diploid parents. Thus, both components of allopolyploidy contribute to their widespread occurrence: (i) interspecific hybridization to trigger changes at all levels from the gene to ecology and (ii) chromosome multiplication to establish these changes. We now face the challenge to determine how perturbations to the transcriptome, metabolome, and proteome influence the phenotype and ecology, requiring the transfer of molecular technologies to an ecological setting. Furthermore, better understanding is needed to explain how polyploids establish fertility, necessitating the search for mechanisms driving regular bivalent pairing; certainly if controlling genes are discovered they may find applications in the production of new polyploid crops.

Conclusions and Future Prospects

In angiosperms, allopolyploidy triggers a new steady state for the transcriptome, metabolome, and proteome networks and perhaps enhances network robustness and/or increases network complexity. We suggest it is genomic plasticity that enables step changes in the steady state, equipping newly originated polyploid individuals with novel biochemical pathways and transgressive characters and allowing them to exploit new niches and/or outcompete diploid parents. Although most new polyploid individuals will die, they must have arisen sufficiently often and with sufficient success to have been a major driving force behind the divergence of angiosperms.

Models explaining how genomic reorganization and epigenetic reprogramming causes changes in gene expression after polyploidy are focused on mechanisms that establish locus-specific expression of duplicated alleles in early polyploids (*9*). Such models need to be tested against rigorous statistical analyses of networks of molecular interactions to determine network robustness and the nature and strength of individual links. Network analyses will also reveal how newly formed polyploids differ in their response to the environment compared with their progenitors and provide understanding of the ecological advantages of polyploidy. We will need to distinguish between responses to polyploidy that are specific, stochastic, or ubiquitous at the levels of individuals, populations, and species. Such information will assist crop breeders and lead to an enhanced understanding of polyploid-generated angiosperm diversity.

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